Comparison of different scaffold materials and different cell types in a 2D+ bioreactor system by LiMSy, a Live cell Monitoring System

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INTRODUCTION

The use of a **live cell monitoring system (LiMSy)** (Fig.1) is a valuable tool to investigate cell dynamics in real-time for various application domains. As a proof of principle for this approach a 2D+ perfusion bioreactor system combined with sensor technology is being used to investigate the influence of scaffold material and cell type in a comparative study.

MATERIALS & METHODS

Human periosteum-derived cells (hPDCs) and **immortalized human bone marrow stem cells (ihBMSC)** were seeded on either 2D+ titanium (**Ti**) or tantalum (**Ta**) scaffolds and cultured statically or dynamically (perfusion). The influence of scaffold pretreatment, cell type and initial seeding conditions (such as seeding time, seeding volume and initial cell density), on cell viability and proliferation capacity were investigated in a static environment. The metabolic activity (Presto Blue metabolic assay) was measured after 1, 5 and 8 days of culture. The oxygen consumption was determined in function of the flow rate. For the latter, a spot (PreSens) and needle (Ocean Optics) oxygen sensor were implemented (Fig.1). In parallel, endpoint analyses such as DNA measurements were carried out as a control.

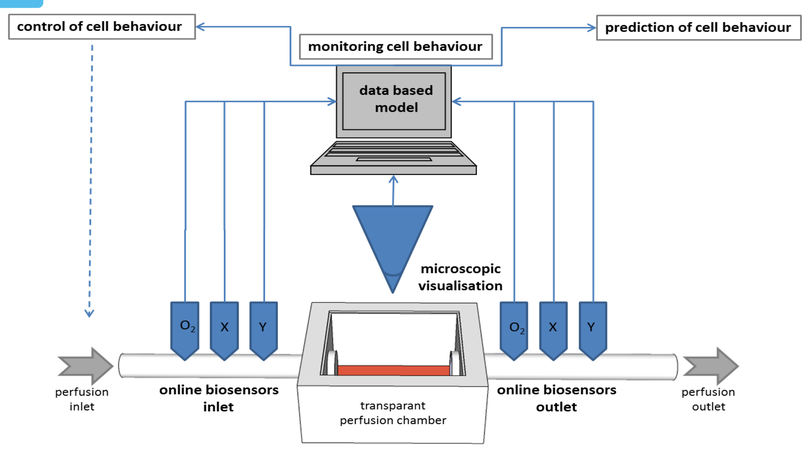
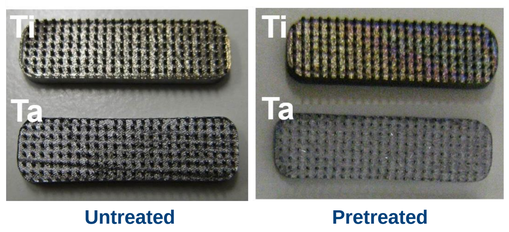


Figure 1. Schematic overview of live cell monitoring system (LiMSy).

RESULTS

How does scaffold pretreatment affect material characteristics and cell behavior?

Figure 2. Pretreatment changes the surface properties for Ti and Ta scaffolds, influencing the initial cell seeding condition. Images of untreated (left) and pretreated (right) 2D+ Ti (↑) and Ta (↓) scaffolds show that white crystals were formed at the surface of Ta scaffolds after treating the scaffolds with 5M NaOH.



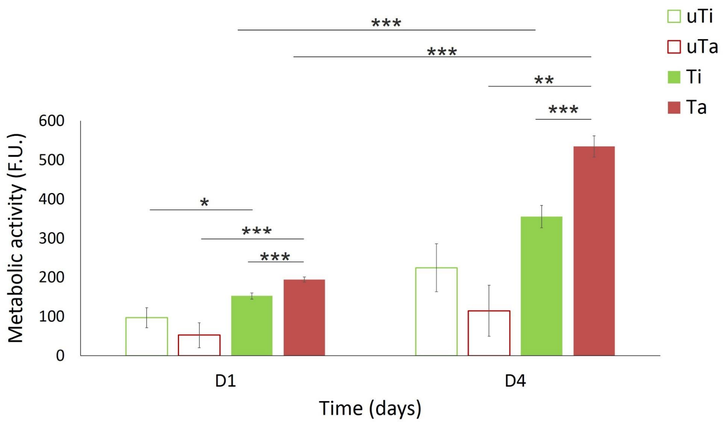


Figure 3. Pretreatment enhances cell attachment and viability in Ti and Ta scaffolds. Untreated and pretreated Ti and Ta scaffolds were drop-seeded with 100 µl cell suspension containing 0.012 x 10^6 cells and cultured statically. Cell metabolic activity was measured by presto blue assay and expressed in fluorescence units (F.U.). Significance level: * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Figure 4. Initial cell attachment and subsequent cell proliferation on 2D+ Ti-scaffolds is dependent on cell type. 0.020 x 10^6 cells (hPDC and i-hBMSC) were drop-seeded on 2D+ Ti scaffolds and DNA measurements were performed at day 1, 5 and 7 and correlated to cell number. Significance level: * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

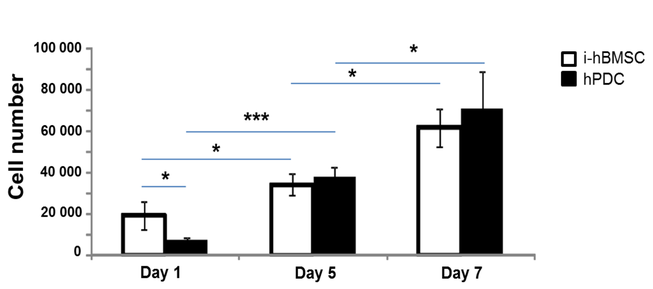


Figure 5. The initial seeding density is the main factor influencing proliferation over time for Ti scaffolds, whereas in case of Ta scaffolds this is the initial seeding volume. Influence of initial seeding parameters (time, seeding volume, and cell density) on cell behavior (metabolic activity) was analyzed using a DOE approach. Pareto charts represent the influence of those factors on the metabolic activity of cells seeded on Ti (A) and Ta (B) constructs, analyzed after 1, 5 and 8 days of culture. The table shows a change over time and a different pattern of critical factors between the two materials. (red line: $p<0.05$)

(A) Ti	Factor	Value	Pareto chart
PB D1	Density	+24,34809	
	Volume	+14,22725	
	Time	+6,168499	
PB D5	Density	+6,415783	
	Time	-3,40914	
	Volume	+2,582413	
PB D8	Time	-5,10748	
	Density	+4,703562	
	Volume	+2,499478	

(B) Ta	Factor	Value	Pareto chart
PB D1	Density	+9,338132	
	Volume	+6,230799	
	Time	+2,059761	
PB D5	Volume	+3,378279	
	Density	+2,239818	
	Time	-0,183891	
PB D8	Volume	+2,424621	
	Time	-0,444401	
	Density	+0,1675073	

How sensitive are the oxygen sensors in terms of change in oxygen consumption based on different flow rates?

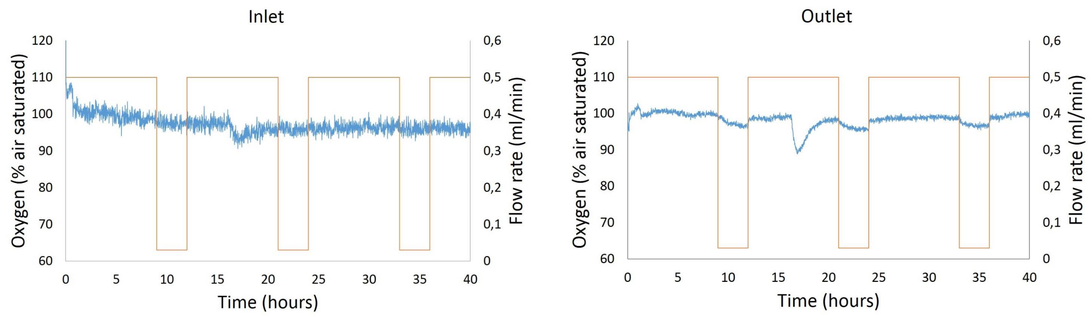


Figure 6. Oxygen consumption in a 2D+ bioreactor system correlates with cell number. Oxygen consumption of cells seeded on Ti scaffolds was determined by measuring the oxygen concentration at the outlet of the bioreactor using a needle (Ocean Optics) oxygen sensor. The flow rate was altered between 0.03 ml/min and 0.5 ml/min as indicated. Oxygen consumption correlated with cell numbers according to $\Delta O_2 = [(Zmax,cell \times cell\ amount) / flow\ rate] \times k_H \times 100$ [1]. To evaluate the constant re-oxygenation, the oxygen levels were measured using a spot sensor (Presens).

CONCLUSION AND DISCUSSION

In this study, we provided evidence that cell proliferation and viability vary between different scaffold materials in a 2D+ bioreactor system and that the oxygen consumption could be correlated to different flow rates applied to a perfusion system. In a next step, these data will be combined with data-based modeling and algorithms will be developed to describe cell behavior of cell populations seeded on different materials. Together, this will lead towards the development of a live cell monitoring system for various applications.

REFERENCES

[1] Truscello, S. et al. 2011. A computational tool for the upscaling of regular scaffolds during in vitro perfusion culture. *Tissue engineering. Part C, Methods*. 17, 6 (Jun. 2011), 619 – 630.

CONTACT DETAILS

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